15.

Agglutinins and Agglutinogens in the Blood of Wild Animals.

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INTRODUCTION.

Through the use of serology, a number of authors (Irwin & Cole, 1936 a, b; Irwin, Cole & Gordon, 1936; Zuckerman & Suderman, 1935; Boyden, 1934; Nutall, 1904) have been able to show phylogenetic relationships between animals of various groups, giving further support to the Linnaean method of classification. In other instances, previously unknown factors were discovered in the blood of animals which have been of extreme importance in the clinical and medico-legal fields (Landsteiner & Wiener, 1940; Landsteiner & Levine, 1928).

Three methods are used in the study of blood groups: Immune serum prepared for the blood cells of one animal is tested with the blood cells of another related or unrelated animal; blood cells and serum belonging to the same species are cross-typed to determine the presence of isoagglutinins; and cells and serum of different species are cross-typed to determine whether heteroagglutinins are present.

With human cells, the presence of group-specific agglutinins may be determined. Such studies might reveal factors common to the blood of man and other animals. The importance of these studies is not of taxonomic interest alone, since similar investigations led to the discovery of important human blood factors (Landsteiner & Wiener, 1940) which were later found related to *erythroblastosis fetalis*, a hemolytic jaundice of the newborn.

Early methods of systematic serology utilized the ring precipitin test (Zuckerman & Suderman, 1935; Boyden, 1934; Nutall, 1904). Further refinements of this test (Boyden & DeFalco, 1943; Boyden, 1943; DeFalco, 1942) involved the use of the photoelectric nephelometer to measure the volume of precipitate formed.

Later investigators used cross-agglutination tests with the blood cells of one animal and the serum of another. This was a simple test that copied the cross-agglutination technics of blood typing and was used to study guinea pigs, mice (Boyd & Walker, 1934; McDowell & Hubbard, 1922) and rats (Rhodenburg, 1919). None of these investigations revealed evidences of isoagglutinins in any of these animals. Studies on rabbits (Levine & Landsteiner, 1931; Levine & Landsteiner, 1929; Snyder, 1924) showed that normal isoagglutinins were absent from such animals when tests were conducted at 37° C., but such agglutinins could be demonstrated after repeated transfusions between rabbits of the same species.

Normal isoagglutinins were found in chickens (Landsteiner & Levine, 1932; Karschner, 1928 b). The former authors used normal chicken serum and the latter used normal ox serum to differentiate the groups.

Isoagglutinins found in horses (Herman, 1936) could be used to divide the animals into definite blood groups similar to those found in humans. Relationship between the horse and closely related species was investigated by Walsh (1924) while studies of bovines (Little, 1929; Karschner, 1928 a) showed the presence of ill-defined groups in these animals.

Irregular heteroagglutinins and isoagglutinins were demonstrated in certain members of the Reptilia (Bond, 1939; 1940 a, b).

Studies of heteroagglutinins for the blood of man revealed the presence of weak agglutinins for all four groups of human cells in the sera of rabbits (Stuart, Sawin, Wheeler & Battey, 1936; Friedenreich & With, 1933; Hooker & Anderson, 1921) while specific agglutinins for certain human blood groups have been found in bovines (Karschner, 1928 a), chickens (Karschner, 1928 b), reptiles (Bond, 1939; 1940 a, b) and various monkeys (Buchbinder, 1933; Landsteiner & Miller, 1925 a, b, c).

Through the use of specially adsorbed rabbit immune serum, other blood factors have been discovered. These have been named P (Landsteiner & Levine, 1931 b), M (Wheeler & Stuart, 1939; Wiener, 1938; Landsteiner & Wiener, 1937; Landsteiner & Levine, 1928; 1931 a) and Rh (Landsteiner & Wiener, 1940).

In relation to the large number of species of animals, and especially mammals, only a relatively few have been examined from the serological point of view. Since there is access to exotic species of mammals in our great zoological gardens, it was thought worth while to make an attempt to study the blood groups in captive wild animals.

The present investigation was carried out to determine whether any agglutinins exist-

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ed, in the sera of certain captive wild and domesticated animals, for the blood cells of man. Where agglutinins for human cells were found, agglutinin adsorption tests were performed to ascertain whether these agglutinins were heterogenetic or specific for any human blood group.

Human sera was also used against the blood cells of different animals to see if such sera had agglutinins for the blood cells of the animals tested. Where such agglutinins were found, it was further determined whether the adsorption of human sera by these blood cells would remove any of the normal human isoagglutinins.

Since the work of Landsteiner & Wiener (1940) has resulted in valuable information on Rh blood groups, work along similar lines is certainly of importance.

Material and Methods.

Blood was collected and allowed to clot. The serum was separated by centrifuging and inactivated for thirty minutes at 37° C. One drop of serum (approximately 0.1 cc.) and one drop of blood cells in 0.85% sodium chloride (approximately 0.5% concentration) were added to a test tube 12 × 75 mm. The tubes were shaken for twenty minutes on a Kahn shaker and were then centrifuged at low speed (1500 R.P.M.) for three minutes. By gentle rocking, the button of cells on the bottom of the tube was dislodged and a reading made.

A firm clot of unbroken clumps was recorded as four plus; the formation of a few smaller clots which were still red on macroscopic examination was recorded as three plus; smaller clots which could be still noticed macroscopically but did not show the definite red color, were recorded as two plus. One plus readings were not clearly visible on macroscopic examination. All specimens giving less than two plus agglutination were read under the microscope at 100 diameters and were designated as one plus, doubtful or negative.

In tests with the Rh sera there was no shaking. The mixture of cells and serum was incubated at 37° C. for two hours, using equal volumes of serum and packed animal cells that were washed three times with 0.85% sodium chloride. The serum and cells were separated by centrifuging and the adsorbed serum was set up against human cells known to agglutinate in non-adsorbed serum of the same type.

Where agglutination occurred between the serum of an animal and human cells, that serum was adsorbed at room temperature for two hours by one-half of its volume of washed, packed human cells of type A, B or O. The adsorbed serum was cross-typed with other human cells to determine whether the anti-human agglutinins had been removed.

Where agglutination took place between the cells of an animal and human serum, that serum was adsorbed by the animal cells in the same manner previously mentioned. The adsorbed serum was then set against other human cells to determine whether the normal human isoagglutinins were adsorbed out by the animal cells.

The blood of two hundred and fifty individuals was tested against the blood of thirteen different animal species to determine whether the human sera contained antibodies for the animal blood cells and whether the human blood cells were agglutinated by the sera of the species used.

The animals were the following: (I) Primates — Diana Monkey (*Cercopithecus diana*), Philippine Macaque (*Macaca irus*), Ring-tail Monkey (*Cebus capucina*), Humboldt's Woolly Monkey (*Lagothrix humboldtii*), Baboon (*Papio* sp), and Chimpanzee (*Pan troglodytes*); (II) Rodentia—Common Albino Rabbit (*Ocyctolagus cuniculus*) and Guinea Pig (*Cavia porcellus*); (III) Carnivora — Puma (*Felis concolor*) and Kinkajou (*Potos flavus*); (IV) Artiodactyla—Dromedary (*Camelus dromedarius*), Dwarf Buffalo (*Anoa depressicornis*) and sheep (*Ovis aries*).

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Results.

Diana Monkey.

The sera of three Diana Monkeys were found to agglutinate human type B cells specifically. This anti-B agglutinin could be adsorbed out of the Diana sera by type B cells only. Adsorption by any other type of human cells failed to remove the B agglutinins.

Three specimens of type O+ human cells tested with the serum from a Diana Monkey gave doubtful agglutination. The serum of the other two monkeys did not clump Group O blood cells.

The agglutination of cells of three Diana Monkeys by human sera varied with the serum used but no definite group pattern was shown. Adsorption of human serum by Diana erythrocytes did not remove the normal isoagglutinins from the human serum. Adsorption of anti-Rh_0 (85%) and anti-Rh′ (70%) sera by Diana blood cells did not remove the Rh antibodies since such adsorbed sera would continue to agglutinate Rh-positive cells of the appropriate type. Anti-Rh′′ (30%) serum was adsorbed with the blood cells of

only one Diana Monkey and this adsorption also failed to remove the anti-Rh'' antibodies.

Philippine Macaque.

The serum of one Philippine Macaque was tested against the blood of nineteen individuals. Only the cells of Groups A and AB were agglutinated. Adsorption of the serum by Group A cells removed all of the agglutinins for human cells.

Woolly Monkey.

The serum of one Woolly Monkey was set up against a number of human cells of all types. In no case was any human cell agglutinated. The adsorption of anti-Rh_0 (85%), Rh' (70%) and Rh'' (30%) sera by the blood cells of the Woolly Monkey failed to remove the Rh antibodies.

Ring-tail Monkey.

The sera of three Ring-tail Monkeys agglutinated all human cells with which they were tested but not with equal intensity. Cells of Group A and AB were more strongly agglutinated than cells of the other two groups. Adsorbing these sera either with Group O or Group B blood cells removed the agglutinins for Groups O and B but not for Group A. Adsorption by Group A blood cells removed agglutinins for all groups. Thus there are two antibodies in the serum of the Ring-tail Monkey, a heteroagglutinin for all human cells and a specific anti-A agglutinin.

The cells of four Ring-tail Monkeys were agglutinated strongly by the sera of forty-eight individuals. Adsorbing normal human sera with Ring-tail Monkey cells did not remove the normal human isoagglutinins, since such adsorbed sera still agglutinated cells of the appropriate blood groups. Adsorption of anti-Rh_0 (85%), anti-Rh' (70%) and anti-Rh'' (30%) sera with Ring-tail cells also failed to remove the Rh agglutinins from these antisera.

Baboon.

The serum of one Baboon agglutinated the cells of all human blood groups with which it was tested. Another serum gave doubtful agglutinations with one specimen of O— and one specimen of A— cells. Adsorption experiments showed that one Baboon serum lost all of its agglutinins after adsorption with cells of Groups O—, A— or B—. The other Baboon showed agglutinins for B— cells after being adsorbed with O— cells and weak A agglutinins after being adsorbed by cells of Group A.

All specimens of human serum agglutinated Baboon cells strongly, regardless of type. Adsorption of human sera and of anti-Rh_0 (85%), Rh' (70%) and Rh'' (30%) sera by Baboon cells failed to remove the normal agglutinins from such sera.

Chimpanzee.

The serum of one Chimpanzee agglutinated the cells of all human groups except Group O. One specimen of Group AB— also failed to agglutinate. Adsorption of Chimpanzee serum with Group AB cells removed all of the human agglutinins.

Rabbit.

Experiments with Rabbit blood showed that Rabbit serum agglutinated human cells with varying intensity, depending upon the serum and cells used. Thus a specimen of Rabbit serum may agglutinate one sample of type B blood and not another.

Human sera set up against Rabbit cells gave the same type of reaction. The intensity again varied with the cells and serum used and no definite pattern could be shown where agglutination occurred or failed to take place.

The adsorption of human sera by Rabbit cells failed to remove the normal human isoagglutinins from the sera.

The Rabbits used in these experiments were previously used for pregnancy tests. It has been shown (Witebsky & Klendshoj, 1940, 1941) that Group B and Group O specific substances could be isolated from the gastric fluid and saliva of some individuals. It is possible that these substances are present in the urine of some individuals and the injection of urine from Group B or Group O individuals into Rabbits may have increased the anti-human agglutinins in the blood of these animals.

Guinea Pig.

Guinea Pig serum from two animals failed to agglutinate any of the twenty-two specimens of human blood used in the tests.

Guinea Pig blood cells were agglutinated by all of the forty specimens of human sera used. Adsorption of human sera by the Guinea Pig cells failed to remove the normal isoagglutinins present in such sera. Adsorption of anti-Rh_0 (85%), anti-Rh' (70%) and anti-Rh'' (30%) with Guinea Pig cells also failed to remove the anti-Rh agglutinins.

Puma.

The serum of the Puma agglutinated one specimen each of Type A and B, Rh-positive and Rh-negative blood. There was no agglutination of Type O cells. This anti-A agglutinin could be adsorbed out of the Puma serum by Type A human cells only. The anti-B agglutinin could be adsorbed out only by Type B cells. In the case of the B adsorption, a weak agglutinin still remained in the adsorbed Puma serum for one specimen belonging to Group B.

Thus the Puma has a specific anti-A and anti-B agglutinin. No heterogenetic agglutinins were found.

Some specimens of human Type A and O sera agglutinated Puma cells. Type B and AB sera failed to do so.

Kinkajou.

The serum of one Kinkajou failed to agglutinate any of the human cells used. Adsorp-

tion of anti-Rho (85%), anti-Rh' (70%) and anti-Rh'' (30%) serum by the Kinkajou's cells failed to remove the anti-Rh agglutinins.

Dromedary.

The results of testing Dromedary cells with human sera showed that most specimens of sera were capable of agglutinating the erythrocytes. Only an occasional human serum failed to cause clumping. Adsorption of human sera by Dromedary cells failed to remove the normal human isoagglutinins from such sera.

Dwarf Buffalo.

The cells of the Dwarf Buffalo were agglutinated by the sera of eighteen individuals, representing seven human blood groups. Adsorption of these sera by the cells of the Buffalo failed to remove the normal human isoagglutinins present in such sera.

Sheep.

All human cells were agglutinated strongly by the sera of three sheep. The results of the agglutination adsorption tests were, however, erratic.

Adsorption of sheep sera with O— cells removed all of the anti-O agglutinins. In one sheep, adsorption by Group O cells removed the A and B agglutinins for some blood belonging to those groups. Adsorption with A, B or AB cells did not necessarily result in the removal of these agglutinins from the sheep serum. It was not possible to predict whether adsorption by the cells of any one group would remove agglutinating factors for that group, since some cells of the same type were still agglutinated. In some cases, adsorption by cells of one group removed the agglutinins for another group but still left agglutinating factors for different specimens of the same group.

The intensity with which sheep cells were agglutinated by human sera varied with the specimen. The greatest proportion of human sera agglutinated the sheep cells strongly.

Adsorption of human sera with sheep cells failed to remove the normal human isoagglutinins since the adsorbed sera still was capable of agglutinating cells of the appropriate type.

Table 1 is a composite showing the results obtained with human cells and the sera of the various animals used in these experiments. Table 2 shows the results obtained with human sera and animals cells. Table 3 shows agglutinins present in animal sera that are adsorpable with human cells.

Agglutination showing three and four plus reactions are indicated by the signs ++ in the tables. Those giving one plus and two plus reactions are indicated by +. An "O" indicates a negative reaction, a "?" indicates a doubtful reaction. Where the space is blank no tests were made.

Discussion.

Thirteen different species representing four orders of mammals, Primates, Rodentia, Carnivora and Artiodactyla, were studied. Of the animals investigated, the Puma, Dromedary and Dwarf Buffalo have never before been studied for their agglutinin and agglutinogen content.

No new blood factors were determined by the methods used, but agglutinins for human blood cells were present in the sera of most of the animals used. Human sera agglutinated nearly all the cells of the animals used and failure to produce such agglutination did not follow any specific pattern. Whether or not the use of rabbit immune sera would give the same results is not known, but many of the newer blood factors have been discovered by such means.

There were no blood factors common to all species, and members of the same species did not necessarily react in the same manner to human sera. However, using animal sera against human cells, similar results were obtained in some instances when the serum of identical species was used.

The sera of members of the same family, but different genera, did not necessarily act

TABLE 1.

Animal Sera Versus Human Blood Cells.

Human Cells	Diana Monkey	Philippine Macaque	Woolly Monkey	Ring-tail Monkey	Baboon	Chimpanzee	Guinea Pig	Puma	Kinkajou	Sheep
0+	0	0	0	+	?++	0	0	0	0	++
0—	0	0	0		++	0	0	0	0	++
A+	0	++	0	++	?+	++	0	++	0	+++
A—	0	++	0	++	+++	+	0	++	0	++
B+	++	0	0	+++	++	+	0	++	0	++
B—	++	0			++		0	++	0	
AB+		++	0	++	++	0++	0		0	++
AB—	++	++	0	++		++				++

TABLE 2.

Human Sera Versus Animal Blood Cells.

Human Sera	Diana Monkey	Ring-tail Monkey	Baboon	Rabbit	Guinea Pig	Puma	Drome-dary	Dwarf Buffalo	Sheep
0+	++	++	++	++	++	0	+	++	?++
0−	++	++	+++	++	++	0+	++	++	++
A+	0+++	++	++	+++	++	0+	+	++	+++
A−	+++	++	++		++	0++	0++	++	0+++
B+	++	++	++	++	++	0	+	++	+++
B−	0++		++		++	0	++	++	++
AB+	+++	++	++		++		0+	++	++
AB−	++			++					

TABLE 3.

Agglutinins Adsorpable by Human Cells.

Serum of:	Human Blood Cells				Remarks
	0−	A−	B−	AB−	
Diana Monkey	−	−	+		Beta agglutinins present
Philippine Macaque		+			Alpha agglutinins present
Woolly Monkey					No agglutinins present
Ring-tail Monkey	+(1)	+(2)	+(1)		Specific Alpha agglutinins (see below)
Baboon	+(3)	+(3)	+		Heterogenetic agglutinins O,A,B, present in serum of one Baboon (see below)
Chimpanzee				+	Alpha and Beta agglutinins adsorbed by one specimen of AB− cells
Puma	−	+(4)	+(5)		(See below)
Sheep	+	+(6)	+(7)	+(8)	(See below)
Guinea Pig					No agglutinins present
Kinkajou					No agglutinins present
Rabbit					(See 9 below)

(1). These agglutinins are heterogenetic and can be removed by O or B cells.
(2). The agglutinin is specific and removable by type A cells only.
(3). Weak Beta agglutinins in 1 specimen removed only by B blood cells.
(4). Anti-A only removed.
(5). Removed most anti-B, leaving feeble anti-B+. Did not remove anti-A.
(6). Removed anti-A− but not anti-A+.
(7). Removed anti-B+ in some serum specimens but not in others.
(8). Some AB− adsorption removed anti-A, O or B in some specimens, but this adsorption did not occur with all serum specimens.
(9). Heteroagglutinins present. Type present varied with the specimen of serum. The same specimen of serum would agglutinate type B+ cells of one individual and fail to agglutinate type B+ cells of another individual.
A "+" sign indicates that agglutinins present are removable by the blood cells of the type shown.

alike. For example, in the family Cercopithecidae, the two Baboons (*Papio* sp.) showed heterogenetic agglutinins, but one had an anti-B agglutinin in addition to this. Three Diana Monkeys, *Cercopithecus diana*, showed only anti-B agglutinins in their sera, while one Philippine Macaque, *Macaca irus*, showed only anti-A agglutinins. It would be interesting to determine whether members of the same genus would fall into similar groups. Landsteiner & Miller (1925 c) did show that 22 members of 12 species of Platyrrhina all gave strong agglutination with their purified agglutinating solutions prepared from Group II (A) and Group III (B) human sera. In these experiments different results were obtained on identical species. However, the difference was undoubtedly due to the fact that unmodified human sera was used in cross-typing with animal cells. Though agglutination took place in nearly all instances, it was shown by agglutinin adsorption tests that the agglutination was heterogenetic and the blood cells of these animals were incapable of adsorbing out the normal human isoagglutinins from human sera. The agglutinogens in the blood cells of these animals were not identical to those in human cells.

Studies of the Diana Monkey are in complete agreement with those of Landsteiner & Miller (1925 c). No agglutinogens were found in the blood cells of the Diana which resembled those in human blood. All agglutinations were heterogenetic. The serum of the Diana Monkey was able to specifically agglutinate Groups B and AB human cells only. That this was specific and not heterogenetic was shown by the fact that the anti-human red cell agglutinins in the Diana Monkey could be adsorbed only by human Group B cells.

Using purified agglutinating sera, Landsteiner & Miller (1925 c) were able to show that agglutinogens similar to B were absent from the Cercopithecidae. Diana is a member of this family. It would not be expected that a B type agglutinogen would be present in an animal possessing an anti-B agglutinin in its serum.

With the Philippine Macaque, agglutinins were present in the serum for human A and AB cells only. These could be specifically adsorbed out only by Group A cells. Since the A agglutinin is similar to the Forssman heterophile agglutinin (Landsteiner & Miller, 1925 c) and the Macacus is heterophile negative (Buchbinder, 1933) and does contain anti-A or anti-heterophile agglutinin in its serum, the results followed those of the two aforementioned authors.

The sera of two Baboons showed heterogenetic agglutinins for all human cells. One Baboon also had a strong specific anti-B agglutinin which could be adsorbed out only by Group B human cells. A weak anti-A antibody, still present after adsorption with Group A cells but not after adsorption by Groups O or B cells, was probably due to insufficient adsorption with the Group A cells used. It was not possible to repeat this experiment because of the small amount of Baboon serum available at the time of the test. Since Landsteiner & Miller (1925 c) failed to find an agglutinogen similar to human B in the Cercopithecidae, it is possible for an anti-Group B agglutinin to exist within members of this family. This is shown by the Diana Monkey and the Baboon. This agglutinin need not necessarily be present since the Macaque lacks the B antigen and the B agglutinin in its blood.

Agglutinogens similar to the human B were demonstrated in the cells of Platyrrhina (Landsteiner & Miller, 1925 c). On this basis there should not be any anti-B agglutinins in the sera of these animals. In the sera of three Ring-tail Monkeys (*Cebus capucina*) there were two anti-human agglutinins present: a heterogenetic antibody and a specific anti-A agglutinin. The heterogenetic antibody could be adsorbed out of the serum to type O human cells. Such adsorption did not remove the anti-A agglutinin. This could be removed by type A cells alone. The one specimen of the Woolly Monkey also failed to agglutinate any human cells, showing complete absence of heterogenetic or group specific antibodies in its serum.

The serum of one Chimpanzee contained agglutinins for human A and B cells. This would be expected on the basis of the work of Landsteiner & Miller (1925 b) who found that the similarity of Chimpanzee and human blood was so close that adsorption of human B sera with human Group A blood cells also removed the agglutinins for the Chimpanzee blood cells. They classified the Chimpanzee as belonging to groups similar to the human A and O groups. The one Chimpanzee studied here seems to belong to Group O on the basis of the agglutinins present in its serum. These A and B agglutinins were removed by adsorption with human AB cells. Landsteiner & Miller (*loc. cit.*) used purified agglutinating solutions on animal cells. In these experiments, unaltered sera was used. Since agglutinogens similar to human B have been demonstrated in the Platyrrhina (Landsteiner & Miller, 1925 a, b, c), it would not be expected that specific anti-B substances would be found in the sera of these animals. The two species of the genera *Cebus* and *Lagothrix* did not have specific B antibodies in their sera.

Since B agglutinogens were absent in the family Cercopithecidae, it was possible for B antibodies to exist in the sera of members of this family. Two members, *Papio* sp. and *Cercopithecus diana*, had specific B agglutinins while the third member, *Macaca*, lacked the B antibody in its serum.

The work on rabbits confirms the work of Hooker & Anderson (1921), who also demonstrated agglutinins for human cells in the sera of the rabbit. Further agreements were

shown in the greater activity of these sera in their action toward A and B cells than toward O cells. These authors further stated that human isoagglutinin beta in Group I (O) and Group II (A) sera can be adsorbed by rabbit cells, resulting in a fall of the titre of such adsorbed human sera. This adsorption is not complete since undiluted adsorbed human sera still maintains sufficient normal human isoagglutinin to cause strong agglutination with the appropriate cells.

One difference between the experiments of Hooker & Anderson (1921) and those of this paper is that the rabbits used for these experiments had been previously injected for pregnancy tests. It is possible that the injection of human urine into the rabbits may have increased the anti-human agglutinin titre of the rabbit. However, the results obtained are substantially the same as those of Hooker & Anderson (1921).

B agglutinins were found to a greater extent in these experiments than in those of Friedenreich & With (1933). These authors found that a strong anti-B agglutinin was present in the undiluted sera of three of forty-seven rabbits. Our results are not in agreement with those obtained by these authors.

As noted above, the rabbits had been previously injected with human urine. This may have increased the anti-B titre of the rabbit sera where such specimens of urine came from Type B individuals.

The sera of two Gunea Pigs failed to agglutinate any of twenty-two different cells, representing all four human blood groups, although human sera of all groups consistently agglutinated the blood cells of the Guinea Pig.

The Puma was the only member of the Carnivora examined whose serum showed anti-A and B agglutinins. These were specific and could be adsorbed by cells of the appropriate type only. The blood cells of the Puma were agglutinated by three specimens of human sera of seventeen used. Two of these were from human Group A and one from human Group O.

Studies of sheep gave erratic results. All sheep sera agglutinated all specimens of human blood cells. Adsorption of sheep sera by human blood cells of one group would, in some cases, remove the agglutinins for that group alone, while in other instances it would remove the agglutinins for other groups as well. In other experiments, adsorption by one type of blood cells still left agglutinins for other specimens of the same human blood group. There was no predictable manner in which the sera would respond to agglutination and agglutinin adsorption tests with human cells. Possibly sheep serum contains agglutinins for sub-groups so that adsorption by one type of human blood cell still left agglutinins for certain of these sub-types.

The action of human sera on sheep cells varied from strong agglutination to no agglutination. No relation could be demonstrated between the blood group and the result of the agglutination test. The sheep cells did not remove the normal human isoagglutinins from human sera, since such adsorbed sera was still capable of agglutinating cells of the appropriate type.

Some phylogenetic significance is shown since the monkeys used in these experiments fall into two categories: those possessing an anti-B agglutinin and those lacking it. Those with the B agglutinin in their blood are members of the Cercopithecidae. However, the absence of this antibody for human Group B cells did not necessarily preclude membership in a different family.

The new world monkeys, *Cebus* and *Lagothrix*, did not have an anti-B agglutinin. Since agglutinogens similar to Type B were demonstrated in their cells by Landsteiner & Miller (1925 c), such antibodies would not be expected.

The *Macaca*, which did not have an anti-B agglutinin, did have a Group A antibody. It is interesting to note that Buchbinder (1933) mentions this anti-A agglutinin in *Macaca rhesus* and the same antibody has been found here in *Macaca irus*. Though two species are not enough from which to draw conclusions, it would be of interest to determine whether all members of the same genus have the same anti-human agglutinins in their blood.

Approaching the problem of systematic serology from a different point from that taken by Boyden (1934, 1942), who performed precipitin tests with immune and normal serum of related species, and having cognizance of the small number of animals examined in these experiments, the results obtained with Primate sera and human cells support the views of Boyden (1942) that serology may be used to investigate the "generally accepted principles of systematic zoology" (Boyden, *loc.cit.*).

It is possible to classify the animals studied, especially the Primates, into groups on the basis of the presence or absence of anti-human red cell agglutinins in their sera. The presence of anti-human Group B agglutinin seems to correspond to membership in the family Cercopithecidae, though the absence of this B antibody does not exclude membership in the same family.

The presence of an anti-human Type A agglutinin in the blood sera of the primates does not lend itself as easily for use in the classification as does the presence of the anti-B. Two members of the genus *Macaca*, *M. irus* and *M. rhesus*, both have A agglutinins in their sera while three different specimens of Ring-tail Monkey, *Cebus capucina*, also possessed specific human A agglutinins.

The use of modified agglutinating solutions of Landsteiner & Miller (1925 c) and unmodified sera as used in the present investigations could be added to the procedure of Boyden (1934, 1942) using immune animal sera.

Conclusions.

Within the limits of the technic and the animals used, the following results were obtained:

The intensity with which the serum of an animal agglutinates the cells of another related or unrelated animal is a characteristic of the individual specimen. With human sera there was no relation between the blood group and the avidity with which it agglutinated animal cells.

It is not possible to classify into blood groups the animals studied, using the agglutination of their blood cells by human sera as a criteria.

Normal isoagglutinins in human sera cannot be completely adsorbed out by the blood cells of the animals studied nor can the anti-Rh factors in human anti-Rh sera be removed by similar technics. The Rh factor was discovered through the injection of rabbits with *Macaca rhesus* blood cells. This agglutinogen is absent in the blood cells of the monkeys studied, as indicated by the fact that anti-Rh$_0$, anti-Rh' or anti-Rh'' did not lose their agglutinins after adsorption with the blood cells of the monkeys used in these studies.

The agglutination of human blood cells by the sera of the animals used is either group specific, heterogenetic or a combination of both factors. These specific human blood cell agglutinins can be adsorbed out of the animal sera with human erythrocytes of the appropriate type. This is the first time that studies have been made with known Rh-positive and Rh-negative cells. None of the animals used in these experiments had normal agglutination antibodies to Rh.

On the basis of agglutinins for human blood cells in their sera, it is possible to assign the animals studied in these experiments to groups: anti-B group—Diana Monkey and Baboon; anti-A group—Philippine Macaque and Ring-tail Monkey; anti-AB group – Chimpanzee and Puma. Another group consisting of those possessing agglutinins for all human blood groups and unclassifiable types will include the rabbit and sheep. Since some of the animals studied, the Philippine Macaque and Diana Monkey for example, possess agglutinating antibodies for only one type of human blood group, it is possible to use the sera of such animals for typing human blood. Those animals possessing agglutinins for more than one human blood group may, in some instances, be used for blood typing if they are selectively adsorbed by appropriate human cells before use.

Summary.

The blood cells and sera of humans were cross-typed with the blood cells and sera of thirteen different animals species. Where agglutination occurred, agglutinin adsorption experiments were carried out to determine whether such agglutination was heterogenetic or group specific.

In eight of the eleven animal sera studied, agglutinins for human blood cells were found. The presence or absence of these agglutinins could be used to classify the animals into groups.

In most cases human sera agglutinated the blood cells of the animals studied. Failure to cause agglutination, or the intensity of the reaction, was in no way related to the human blood group of the serum used.

Insofar as is known, this study reports the use of Rh-negative and Rh-positive cells for the first time in the examination of the sera of the animals used in the experiment. Th presence of specific anti-A and anti-B agglutinins in the Puma is also reported for the first time.

The blood cells of the animals used in these experiments cannot adsorb out normal human isoagglutinins or anti-Rh factors from human serum. The methods used in these studies did not reveal the presence of an Rh-agglutinogen in the species utilized.

The sera of some of the animals showing specific anti-human red cell agglutinins can be used for typing human blood.

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